

shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Fourteen days or more after the final dose of vaccine the vaccinates and the controls shall be challenged with virulent canine parvovirus furnished or approved by Animal and Plant Health Inspection Service and the dogs observed each day for 14 days. Rectal temperature, blood lymphocyte count, and feces for viral detection shall be taken from each dog each day for at least 10 days postchallenge and the presence or absence of clinical signs noted and recorded each day.

(i) The immunogenicity of the Master Seed shall be evaluated on the following criteria of infection: temperature  $\geq 103.4^{\circ}\text{F}$ ; lymphopenia of  $\geq 50$  percent of prechallenge normal; clinical signs such as diarrhea, mucus in feces, or blood in feces; and viral hemagglutinins at a level of  $\geq 1:64$  in a 1:5 dilution of feces or a test of equal sensitivity. If at least 80 percent of the controls do not show at least three of the four criteria of infection during the observation period, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing more than one criterion of infection described in paragraph (c)(3)(i), of this section, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Five susceptible dogs (four vaccinates and one control) may be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (c)(1) of this section.

(i) Each vaccinee shall be administered a predetermined quantity of vaccine virus as provided in paragraph (c)(2) of this section.

(ii) Fourteen to 21 days after the last vaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine parvovirus in the same manner used to determine susceptibility.

(iii) If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If three of the four vaccinates in a valid test do not develop titers of at least 1:16 final serum dilution, and the remaining vaccinee does not develop a titer of at least 1:8, the Master Seed is unsatisfactory, except as provided in paragraph (c)(4)(v) of this section.

(v) If the results of a valid SN test are unsatisfactory, the vaccinates and the control may be challenged as provided in paragraph (c)(3) of this section. If at least three of the four criteria of infection are not shown in the control dog, the test is inconclusive and may be repeated, except that if any of the vaccinates show more than one criterion of infection, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine used in the immunogenicity test in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer of  $10^{0.7}$  greater than that used in the immunogenicity test, but not less than  $10^{2.5}$  ID<sub>50</sub> per dose.

[50 FR 436, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

EFFECTIVE DATE NOTE: At 72 FR 72564, Dec. 21, 2007, § 113.317 was amended by removing paragraph (c)(4) and redesignating paragraph (c)(5) as paragraph (c)(4), effective Jan. 22, 2008.

#### § 113.318 Pseudorabies Vaccine.

Pseudorabies Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has

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been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five pseudorabies susceptible pigs (20 vaccinates and 5 controls) of the youngest age for which the vaccine is recommended, shall be used as test animals. Blood samples shall be taken from each pig and the serums inactivated and individually tested for neutralizing antibody against pseudorabies virus. Pigs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test using 50 to 300 TCID<sub>50</sub> pseudorabies virus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 pigs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) Fourteen to 28 days postvaccination, the vaccinates and controls shall be challenged with virulent pseudorabies virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days.

(i) If at least four of the five controls do not develop severe central nervous system signs or die, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates in a valid test do not remain free of signs of pseudorabies, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot is discontinued. Only five vaccinates and five controls need to be

used in the retest. Susceptibility and age requirements shall be as provided in paragraph (b)(1) of this section.

(ii) Fourteen to 28 days postvaccination, a blood sample shall be taken from each pig and the serum inactivated and tested for neutralizing antibody to pseudorabies virus by the same method used to determine susceptibility.

(iii) If the five controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(iv) If at least four of the five vaccinates in a valid test have not developed titers of 1:8 final serum dilution or greater and the remaining vaccinate a titer of 1:4 or greater, the Master Seed is unsatisfactory, except as provided in paragraph (b)(4)(v).

(v) If the results of a valid neutralization test are unsatisfactory, the vaccinates and controls may be challenged as provided in paragraph (b)(3) of this section. If at least four of five controls do not develop severe central nervous system signs or die, the test is inconclusive and may be repeated. If all five of the vaccinates in a valid test do not remain free of signs of pseudorabies, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph.

(2) *Virus titer requirements.* Final container samples of completed product shall be titrated by the method used in paragraph (b)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer at least 10<sup>0.7</sup> greater than that used in the immunogenicity test, but not less than 10<sup>2.5</sup> TCID<sub>50</sub> per dose.

[50 FR 437, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

EFFECTIVE DATE NOTE: At 72 FR 72564, Dec. 21, 2007, §113.318 was amended by removing paragraph (b)(4) and redesignating paragraph (b)(5) as paragraph (b)(4), effective Jan. 22, 2008.

**§§ 113.319–113.324 [Reserved]**

**§ 113.325 Avian Encephalomyelitis Vaccine.**

Avian Encephalomyelitis Vaccine shall be prepared from virus-bearing tissues or fluids from embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe, and immunogenic in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Avian encephalomyelitis susceptible chickens, all of the same age (eight weeks or older) and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an ali-

quot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed ten-fold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inoculate at least 10 embryos, 5 or 6 days old, in the yolk sac with 0.2 ml each. Twenty similar embryos obtained from the same source shall be kept as uninoculated negative controls. Disregard all deaths during the first 48 hours post-inoculation.

(ii) Eggs for each dilution shall be kept in separate containers and allowed to hatch. Sufficient precaution shall be taken to assure that chickens from each dilution remain separated. To be a valid test, at least 75 percent of the uninoculated eggs shall hatch.

(iii) On the third day after normal hatching time, count all unhatched eggs and all dead, paralyzed and ataxic chickens as positive evidence of viral infection.

(iv) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(v) Calculate the  $EID_{50}$  by the Spearman-Kärber or Reed-Muench method.

(3) At least 21 days post-vaccination, the vaccinates and the controls shall be challenged intracerebrally with a virulent avian encephalomyelitis virus and observed each day for 21 days.

(4) If at least 80 percent of the controls do not show signs of avian encephalomyelitis or die, the test is inconclusive and may be repeated. If at least 19 of 20, or 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free from clinical signs of avian encephalomyelitis during the observation period, the Master Seed Virus is unsatisfactory.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(4) of this section.